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DIRECT MEASUREMENT OF DIRECTIONAL DISORDER FOR CILIARY METACHRONAL WAVE

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Abstract-**The interrelationship of cilia and the directional disorder of ciliary metachronal wave were analyzed using digital microscopic images. The degree of synchronization between ciliary beats was determined by the correlation factor between two different spots. Principal axes of inertia were applied to find out the uniphase directions of beating cilia. The standard deviation of wave directions in an ROI was defined as a measure of ciliary wave disorder (CWD). The pooled mean of CWD was 22.9+/-8.73° in ROIs of 8μm×8μm and 25.4+/-6.46° in 32μm×24μm from the sphenoid sinus mucosa of 5 normal subjects. Our result shows that there is a considerable variation in metachronal wave directions of cilia beating on the epithelium.**

Keywords - **Digital microscopic images, correlation map, uniphase directions of cilia, principal axis of inertia, ciliary wave disorder (CWD).**

I. INTRODUCTION

The main task of mucociliary systems in the respiratory tract is to eliminate inhaled particles by the propulsion of mucus. The overall propulsive effect depends upon the arrangement of the cilia, their metachronal relationships, as well as on the ciliary beat frequency (CBF) [1]. Some methods based on image analysis allow the determination of the CBF of a single cell or multiple ciliated cells simultaneously [2]-[4]. Disorientation of cilia leads to an impairment of mucociliary transport. To determine the coordination of ciliary beating, variations in the orientation of cilia have been previously measured by examining cross-sections of the epithelium using an electron microscope [5]. It has been found that ciliary disorientation may occur secondary to inflammation caused by infection [6] and that the measured orientation of cilia may vary in normal subjects [6]-[8]. Ciliary disorientation alone can lead to the clinical syndrome of primary ciliary dyskinesia (PCD) [9]. The directions based on observations of ciliary cross-sections may differ from the metachronal wave directions of cilia beating in media. We have applied principal axis of inertia to the 2-dimensional correlation map calculated from sequential ciliary images. This method directly measures the phase relations and wave directions of multiple cilia beating in culture media.

II. MATERIALS AND METHODS

The mucosa in the sphenoid sinus were collected from subjects of no respiratory diseases with informed consent. The ciliary movements were recorded using a CCD camera from an inverted microscope. A personal computer equipped with an image-grabber [IC-Comp ; Imaging Technology , Inc.] converted VCR signals into digital images with a depth of 8 bits. Under the magnification used, one pixel size equated to 0.1μm. The digitized images for each processing were acquired at 30Hz for about 4.27 seconds (128 frames). The whole field of 640 by 480 pixels was divided into equal sized blocks (spots) of 2 by 2 pixels. Gray-level values of all pixels in each spot were summed sequentially from the first frame to the last.

Movements of cilia are coordinated in time and space by phase differences, and this is called metachronism or a metachronal wave [10]. The Pearson correlation factor between series from two different spots determined the degree of synchronization between ciliary beats. The correlation values represent the phase differences relative to the reference spot exactly, if the series considered are of the same frequency. The maximal phase gradient is perpendicular to the line of synchrony or uniphase [11]. The principal axes of inertia were applied to determine a uniphase line of beating cilia with zero phase difference [12]. The principal axis of the minimum moment of inertia was estimated as the uniphase direction using correlation factors from a rectangular neighborhood (Eq. 1). The direction of the wave propagation was defined as being perpendicular to the uniphase lines [10]. The angles representing directions of wave propagation were in the range of 180°. Presuming that all the cilia in the spot beat in the same direction, a distribution map of directions was composed. To analyze the distribution of the measured wave directions in a specific part of a microscopic field, the area concerned (the region of interest or ROI) was selected as a rectangular window. The standard deviation describing variations in the directions was regarded as an overall measure of ciliary wave disorder (CWD) for that ROI.

$$x_0 = \frac{1}{(2N+1)^2}\sum_{x=-N}^{N}\sum_{y=-N}^{N} xP(x,y),\ \ y_0 = \frac{1}{(2N+1)^2}\sum_{x=-N}^{N}\sum_{y=-N}^{N} yP(x,y)$$

$$M_{ij} = \sum_{x=-N}^{N}\sum_{y=-N}^{N}(x_0 - x)^i (y_0 - y)^j P(x,y)$$

$$q = \frac{1}{2}\tan^{-1}\left(\frac{2M_{11}}{M_{20}-M_{02}}\right), \quad 0 \le q < 180 \qquad (1)$$

$2N+1$: width of neighborhood in pixels

$P(x,y)$: correlation value between each spot and reference

M_{ij} : ij-th moments

q : principal axis direction of minimum moment of inertia

III. RESULTS

The correlation maps in Fig. 2 show the correlation values between ciliary signals at each spot and the reference around the center of an image. The pixels shown as gray levels represent negative correlations while the color-coded pixels stand for positive correlations. Color and gray stripes repeat in one direction. These alternating patterns in specific directions were discovered for most of the mucosa from the reference although they weakened with distance. These close phasic connections between ciliary beats are essential for the

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Abstract

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effective propulsion of mucus by the metachronal wave of the cilia. The directions of uniphase lines are determined by correlation factors obtained from a neighborhood of 17 by 17 pixels. The black lines at Fig. 2 represent directions of wave propagation, which are perpendicular to the uniphase lines. The maps of Fig. 3 represent visually the wave directions determined by correlation values calculated in each spot. Table I summarizes measurement results of ciliary wave disorder for 5 healthy subjects when the size of ROIs is 8µm×8µm and 32µm×24µm in 5 different fields. The pooled mean of ciliary wave disorder was 22.9+/-8.73° in ROIs of 8µm×8µm and 25.4+/-6.46° in 32µm×24µm. Subjects 4 and 5, with greater local disorder in unit of cells, also showed a higher disorder in global behavior. The results show that there is a considerable variation in the metachronal wave directions of cilia beating on the epithelium.

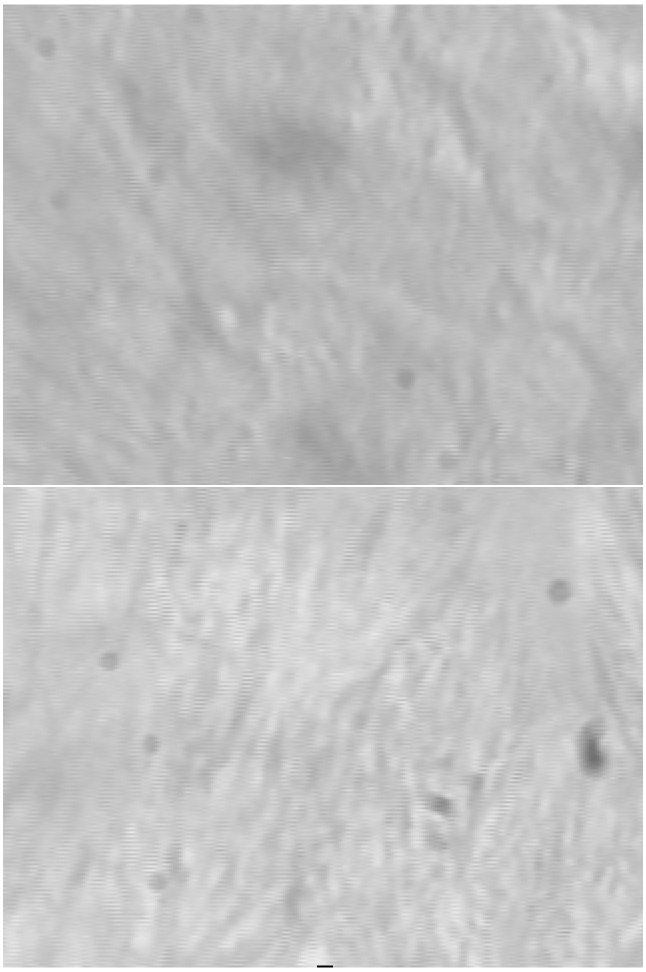

Fig. 1. Digitized gray images of ciliary cells beating in ROIs of 32µm×24µm for 2 different subjects.

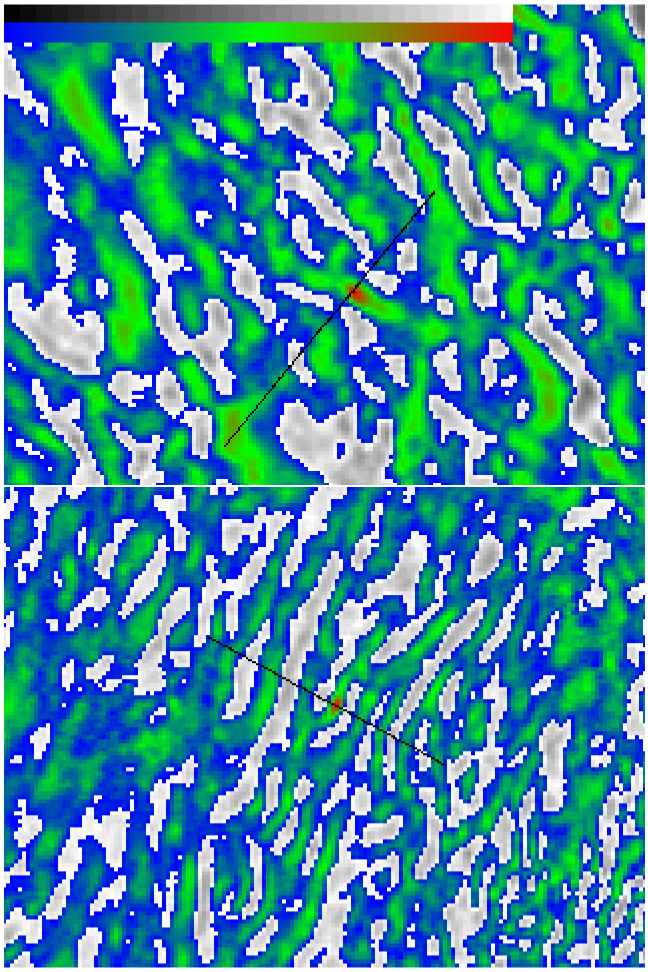

Fig. 2. Correlation maps and lines representing wave directions at the reference spot. Wave direction of upper is 40° and lower 118° (Vertical up direction of the field is 0°, horizontal right 90°, and vertical down 180°).

TABLE I
MEASUREMENT RESULTS OF CILIARY WAVE DISORDER (CWD) AT 8 ROIS OF 32µM×24µM AND AT 96 ROIS OF 8µM×8µM IN 5 DIFFERENT FIELDS FOR EACH SUBJECT.

Subject	*CWD in ROIs of 32mm ´24mm (°)*		*CWD in ROIs of 8mm ´8mm (°)*	
	Mean	SD	Mean	SD
1	18.5	2.60	15.8	4.55
2	24.5	5.78	21.5	7.61
3	25.6	4.87	23.6	7.30
4	30.6	5.99	28.4	9.73
5	30.5	3.67	28.1	6.95

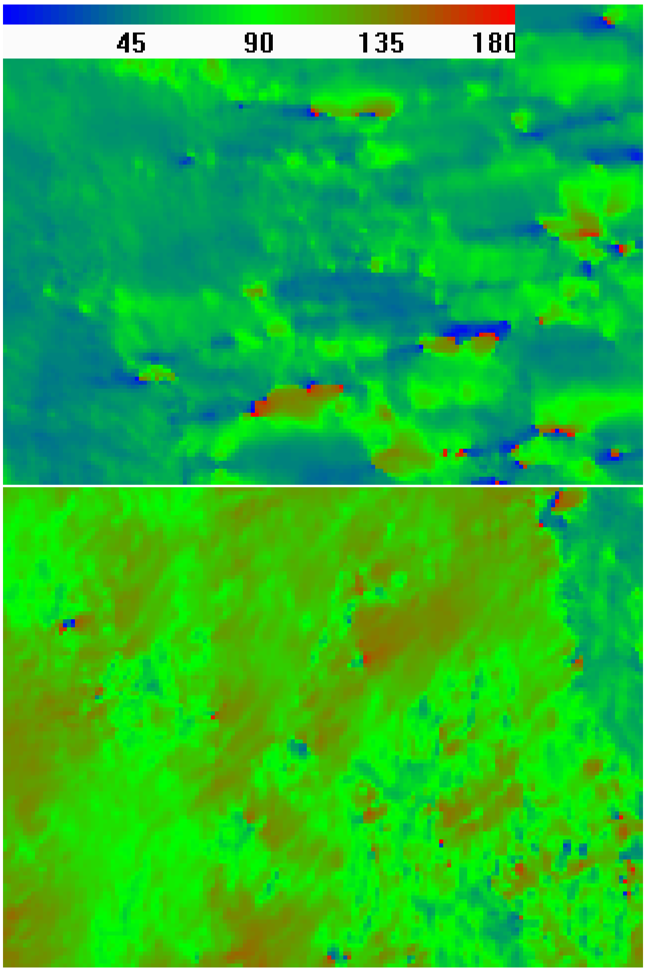

Fig. 3. Direction maps of ciliary wave. Ciliary wave disorder of upper is 18.7° and lower 19.4°. (Blue color represents a wave direction of 0°, green 90° and red 180°).

IV. Discussion and Conclusion

Ciliary orientation was estimated from the micrographs by measuring the angle between the line through two central tubules of cilia and a reference using an electron microscope [5]. The orientation of cilia in normal subjects varied with a disorientation of 14.6+/-3.3° in a study by De Iongh and Rutland [8] and 10.47+/-0.53° by Rayner et al. [6]. Our finding is similar to the result of Rautiainen, in which the disorientation was 23.8+/-6.3° in nasal samples and 29.9+/-7.3° in sphenoid sinuses [7]. The disorientation of PCD patients was 38.7+/-7.8° in a study by De Iongh and Rutland [8] and 23.3+/-1.5° for central pairs and 23.5+/-2.5° for basal feet by Rayner et al [9], all of which were significantly greater than the normal groups. The photoelectric method could simultaneously measure ciliary beat frequency, phase shifts and correlation factors from small active ciliary areas [13]. However, the photoelectric method using optic fibers cannot provide multiple measurements of the wave direction simultaneously. A method capable of measuring the metachronal wave disorder on active ciliary epithelium in vivo or ex vivo has not yet been developed. We believe that our method can evaluate the efficacy of cilia beating on the epithelium more accurately.

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